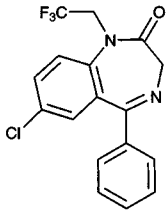
KEY WORDS
use plasticware; plasma; post-column reaction; pharmacokinetics

REFERENCE
Muindi,J.F.; Lee,S.-J.; Baltzer,L.; Jakubowski,A.; Scher,H.I.; Sprancmanis,L.A.; Riley,C.M.; Vander Velde,D.; Young,C.W. Clinical pharmacology of deoxyspergualin in patients with advanced cancer, *Cancer Res.*, **1991**, *51*, 3096–3101.

Halazepam



Molecular formula: $C_{17}H_{12}ClF_3N_2O$
Molecular weight: 352.74
CAS Registry No.: 23092-17-3
Merck Index: 4619

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut C8 SPE cartridge with 2 mL MeOH and 2 mL water, do not allow to dry. Add 100 μL 5 ng/mL diazepam in 1 M pH 10.5 glycine buffer then 1 mL plasma to the SPE cartridge, wash with 2 mL water, wash with 50 μL MeOH, elute with three 200 μL aliquots of MeOH. Combine the eluates and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, inject a 50-80 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Adsorbosphere C8
Mobile phase: MeOH:20 mM pH 4.0 phosphate buffer 60:40
Flow rate: 1
Injection volume: 50-80
Detector: UV 240

CHROMATOGRAM
Retention time: 9.31
Internal standard: diazepam (7.76)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: nordiazepam
Simultaneous: alprazolam, chlordiazepoxide, clonazepam, desmethyldiazepam, 3-hydroxyhalazepam, lorazepam, methylclonazepam, oxazepam, prazepam, quazepam, temazepam, triazolam

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Gupta,S.K.; Ellinwood,E.H. Liquid chromatographic assay and pharmacokinetics of halazepam and its metabolite in humans, *J.Pharm.Sci.*, **1990**, *79*, 822–825.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 2.5 mL Microsomal incubation + 2.5 mL acetone, add 30 μL diazepam in MeOH, add 2.5 mL chloroform, centrifuge. Remove the organic layer and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 6.2 7 μm Zorbax silica

Mobile phase: Hexane:dichloromethane:isopropanol 77:20:3
Flow rate: 2
Detector: UV 232

CHROMATOGRAM
Retention time: 10
Internal standard: diazepam (12)

OTHER SUBSTANCES
Extracted: metabolites, oxazepam

KEY WORDS
human; liver; normal phase; pharmacokinetics

REFERENCE
Lu,X.-L.; Guengerich,F.P.; Yang,S.K. Stereoselective metabolism of prazepam and halazepam by human liver microsomes, *Drug Metab.Dispos.*, **1991**, *19*, 637–642.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenyl-

butazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize brain in 100 mM NaOH (5 mL/g) by sonication. Add 250 μL homogenate (ca. 50 mg tissue equivalent) to 250 μL 100 mM pH 13 NaOH, vortex thoroughly. Add 2 mL toluene, mix (50 inversions), centrifuge at 15000 rpm at 4° for 20 min. Dry the organic phase under a stream of nitrogen. Add 2 mL toluene to the aqueous phase and repeat the extraction. Combine the organic layers and dry under a stream of nitrogen. Reconstitute the residue in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 3 μm Rainin C8 Microsorb
Mobile phase: MeCN:MeOH:25 mM potassium phosphate buffer 18.5:16.5:65
Flow rate: 0.9
Injection volume: 40
Detector: UV 240

CHROMATOGRAM

Retention time: 6.2
Internal standard: halazepam

OTHER SUBSTANCES

Extracted: midazolam

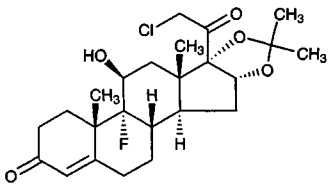
KEY WORDS

brain; rat; halazepam is IS

REFERENCE

Jiang,Q.; Walton,N.Y.; Gunawan,S.; Treiman,D.M. High-performance liquid chromatographic determination of midazolam in rat brain, *J.Chromatogr.B*, **1996**, *683*, 276–280.

Halcinonide



Molecular formula: $C_{24}H_{32}ClFO_5$
Molecular weight: 454.97
CAS Registry No.: 3093-35-4
Merck Index: 4621
Lednicer No.: 2 187

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10-60 μL aliquot.

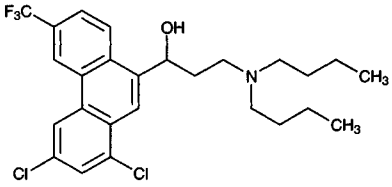
HPLC VARIABLES
Column: 150 × 2 5 μm Spherisorb ODS1
Mobile phase: MeCN:water 50:50
Flow rate: 0.16-0.30
Injection volume: 10-60
Detector: UV 240

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: triamcinolone acetonide

REFERENCE
Gardner,R.S.; Walker,M.; Hollingsbee,D.A. A sensitive high-performance liquid chromatographic method for the assessment of percutaneous absorption of topical corticosteroids, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 1083–1085.

Halofantrine



Molecular formula: $C_{26}H_{30}Cl_2F_3NO$
Molecular weight: 500.43
CAS Registry No.: 69756-53-2, 36167-63-2 (HCl)
Merck Index: 4626
Lednicer No.: 3 76

SAMPLE
Matrix: blood
Sample preparation: Add 1 ml MeCN:EtOH 99:1 to 500 μL erythrocyte pellet, vortex for 1 min, centrifuge at 2000 g for 5 min. Collect the supernatant, evaporate to dryness under nitrogen, reconstitute with 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm AD Chiralpak amylase tris-3,5-dimethyl phenylcarbamate
Mobile phase: Hexane:2-propanol:2-butanol:diethylamine 95:3:2:0.5
Flow rate: 0.3
Injection volume: 20
Detector: F ex 260 em 380

CHROMATOGRAM
Retention time: 16 (+), 24 (-)

Internal standard: (±S)-dichloro-[2-(dibutylamino)methyl]-6-(trifluoromethyl)-9-phenanthrene-methanol (SmithKline Beecham) (21)
Limit of quantitation: 25 ng

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
erythrocytes; chiral; do the extraction procedure in silanized tubes

REFERENCE
Gorichon,E.; Martin,C.; Bangchang,K.N.; Karbwang,J.; Thuiller,A.; Farinotti,R.; Gimenez,F. Chiral chromatographic method to determine the enantiomers of halofantrine and its main chiral desbutyl metabolite in erythrocytes, *J.Chromatogr.B*, **1998**, *712*, 259–262.

SAMPLE
Matrix: blood
Sample preparation: Add 200 µL 2 µg/mL IS to 500 µL plasma. Add 950 µL MeCN, vortex for 1 min, centrifuge at 700 g for 2 min. Add 2-8 mL MTBE, vortex for 2 min, centrifuge at 700 g for 5 min. Remove the upper organic phase and add it to 100 µL 5 mM HCl in MeCN, evaporate under a stream of nitrogen at 35°. Reconstitute the residue with 200 µL MeCN, vortex for 1 min, centrifuge at 700 g for 2 min. Inject a 25 µL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 µm Newguard RP-8 (Perkin Elmer)
Column: 250 × 4.6 5 µm Ultrasphere C8
Mobile phase: MeCN:water 75:25 containing 0.2% sodium dodecyl sulfate (w/v) and 0.2% glacial acetic acid (v/v)
Flow rate: 1.5
Injection volume: 25
Detector: UV 257

CHROMATOGRAM
Internal standard: 2,6-dichloro-6-trifluoromethyl-9-(1-[2-(dibutyl-amino)ethyl])phenanthrene-methanol.HCl (SmithKline Beecham)

KEY WORDS
plasma; dog

REFERENCE
Porter,C.J.H.; Caliph,S.M.; Charman,W.N. Differences in pre- and post-prandial plasma lipid profiles affect the extraction efficiency of a model highly lipophilic drug from beagle dog plasma, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 175–180.

SAMPLE
Matrix: blood
Sample preparation: Add 300 µL MeCN to 100 µL rat plasma with containing IS, vortex. Centrifuge for 2 min and remove the supernatant. Add 200 µL ammonium hydroxide and 2 mL MTBE:hexane 50:50. Vortex for 45 s, centrifuge at 2500 g for 3 min. Evaporate the organic layer to dryness. Add 250 mM (+)-di-O-acetyl L-tartaric acid anhydride in acetic acid:dichloromethane 20:80, heat at 45° for 30 min. Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 C18
Mobile phase: MeCN:25 mM potassium phosphate/sulfuric acid/triethylamine solution 53.5:46.5 containing 1.8 g/L sodium dodecyl sulfate
Flow rate: 1.2
Detector: UV 254

CHROMATOGRAM
Retention time: 19.3 ((+)-enantiomer), 21.7 ((-)-enantiomer)

Internal standard: imipramine (13.8)
Limit of quantitation: 25 ng/mL

KEY WORDS
plasma; rat; derivatization; chiral

REFERENCE
Padovani,P.K.; Timby,D.M.; Wright,M.R.; Kapil,R.P. Quantitative analysis of DMP 851 in rat and dog plasma by liquid-liquid extraction and reverse-phase high performance liquid chromatography with ultraviolet detection (Abstract 3318), *Pharm.Res.*, **1997**, *14*, S568.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 50 μL 4 μg/mL IS + 1 mL MeCN, vortex for 20 s, centrifuge at 1200 g for 10 min, add to a conditioned 2.8 mL 500 mg Bond Elut C8 SPE cartridge, wash with two 1 mL portions of MeCN, elute with 1 mL MeCN:1 M HCl 90:10. Add the eluate to 1 mL 28% ammonium hydroxide and 6 mL dichloromethane, rotate at 15 rpm for 25 min, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 150 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Ultrasphere C8
Mobile phase: MeCN:water 75:25 containing 0.2% sodium dodecyl sulfate and 0.2% glacial acetic acid
Flow rate: 1.5
Injection volume: 25
Detector: UV 257

CHROMATOGRAM
Retention time: 8.3
Internal standard: 2,4-dichloro-9-(2-dibutylamino-1-hydroxy)ethyl-6-trifluoromethylphenanthrene (BL 22312) (11.5)
Limit of detection: 5 ng/mL
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: chloroquine, dapsone, mefloquine, primaquine, proguanil, pyrimethamine, quinine, sulfadoxine, tetracycline

KEY WORDS
serum; treat glassware with 0.2% aquasil; pharmacokinetics; SPE

REFERENCE
Keeratithakul,D.; Teja-Isavadharm,P.; Shanks,G.D.; Webster,H.K.; Edstein,M.D. An improved high-performance liquid chromatographic method for the simultaneous measurement of halofantrine and desbutylhalofantrine in human serum, *Ther.Drug Monit.*, **1991**, *13*, 64–68.

SAMPLE
Matrix: blood
Sample preparation: Dried blood. Allow 500 μL blood to dry on a strip of filter paper. Store at room temperature, protect from dust and sunlight. Add 15 μL 10 μg/mL IS in water to each strip and allow to dry at 37° for 30 min. Cut strip into small pieces, add 1 mL 10 mM perchloric acid, vortex, let stand at room temperature for 5 min, add 2 mL MeCN, vortex at high speed for 30 s, add 1 mL ammonia, mix thoroughly, add 5 mL hexane, vortex at moderate speed for 1 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 120 μL mobile phase, inject a 50 μL aliquot. Whole blood, plasma. 500 μL Whole blood or plasma + 15 μL 10 μg/mL IS in water + 2 mL MeCN, vortex for 30 s, centrifuge at 2000 g for 5 min. Remove the supernatant and add it to 500 μL ammonia, vortex, add 5 mL hexane:diethyl ether 50:50, vortex for 1 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to dryness under a

stream of nitrogen at 37°, reconstitute the residue in 120 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm CN precolumn RP-18 endcapped (Merck)
Column: 250 × 4.6 Hypersil 5 ODS
Mobile phase: MeCN:water:triethylamine 65:35:1 adjusted to pH 4 with orthophosphoric acid
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 8.5
Internal standard: 2,4-dichloro-9-(2-dibutylamino-1-hydroxy)ethyl-6-trifluoromethylphenanthrene (11)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: diazepam
Noninterfering: acetaminophen, chlorcycloguanil, chloroquine, chlorproguanil, cycloguanil, mefloquine, phenobarbital, proguanil, pyrimethamine, quinidine, quinine, sulfadoxine

KEY WORDS
plasma; whole blood; dried blood; pharmacokinetics; diazepam may be used as IS

REFERENCE
Mberu,E.K.; Muhia,D.K.; Watkins,W.M. Measurement of halofantrine and its major metabolite desbutylhalofantrine in plasma and blood by high-performance liquid chromatography: a new methodology, *J.Chromatogr.*, **1992**, *581*, 156–160.

SAMPLE
Matrix: blood
Sample preparation: Condition a 500 mg Bakerbond SPE cartridge with three 3 mL portions of MeCN and three 3 mL portions of solvent. 1 mL Plasma + 3 mL solvent, add to the SPE cartridge, elute with 2 mL solvent. Collect all the eluate and add it to 5 μL 10 μg/mL desipramine hydrochloride in n-hexane:EtOH:2-butanol 93:4.5:2.5, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL mobile phase, inject a 200 μL aliquot. (Solvent was MeCN:triethylamine:EtOH 99:1:1 adjusted to pH 4 with 1 M HCl.)

HPLC VARIABLES
Guard column: 50 × 4.6 10 μm Chiralpak AD amylose tris-3,5-dimethylphenylcarbamate (Daicel)
Column: 250 × 4.6 10 μm Chiralpak AD amylose tris-3,5-dimethylphenylcarbamate (Daicel)
Mobile phase: n-Hexane:EtOH:2-butanol:diethylamine 93:4.5:2.5:0.1
Flow rate: 0.3
Injection volume: 200
Detector: F ex 300 em 380

CHROMATOGRAM
Retention time: 15.5 (+), 18 (-)
Internal standard: desipramine (32)
Limit of quantitation: 6 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE; plasma; pharmacokinetics; chiral

REFERENCE

Terefe,H.; Blaschke,G. Direct determination of the enantiomers of the antimalarial drug halofantrine and its active metabolite N-desbutylhalofantrine in human plasma, *J.Chromatogr.B*, **1994**, *657*, 238–242.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 30 μL 100 μg/mL IS in MeCN:water 80:20, vortex at high speed and add 2 mL MeCN, centrifuge at 1800 g for 3 min. Remove the supernatant and add it to 500 μL ammonium hydroxide and 5 mL MTBE:hexane 50:50, vortex at high speed for 90 s, centrifuge at 1800 g. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 25°, reconstitute the residue in 300 μL 250 mM (+)-di-O-acetyl-L-tartaric acid anhydride in acetic acid:dichloromethane 20:80 (freshly prepared), heat at 45° for 30 min, add 300 μL MeOH, evaporate to dryness under a stream of nitrogen at 25°, reconstitute with 170 μL mobile phase, inject a 30-100 μL aliquot.

HPLC VARIABLES

Guard column: Guard-Pak ODS (Waters)

Column: 250 × 4.6 Ultrasphere ODS

Mobile phase: MeCN:buffer 53.5:46.5 containing 0.9 g/L sodium dodecyl sulfate (Buffer was 25 mM KH_2PO_4 containing 1.5 mL/L 2 M sulfuric acid and 0.5 mL/L triethylamine, pH 5.0.)

Flow rate: 1.2

Injection volume: 30-100

Detector: UV 254

CHROMATOGRAM

Retention time: 11.5 (+), 13.3 (-)

Internal standard: (±)-2,4-dichloro-α-[2-(dibutylamino)ethyl]-6-(trifluoromethyl)-9-phenanthrenemethanol (SK&F 99123) (17.0, 20.7 (enantiomers))

Limit of quantitation: 12.5 ng/mL

KEY WORDS

plasma; derivatization; pharmacokinetics; chiral

REFERENCE

Brocks,D.R.; Dennis,M.J.; Schaefer,W.H. A liquid chromatographic assay for the stereospecific quantitative analysis of halofantrine in human plasma, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 911–918.

SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL 200 mg Bond Elut C8 SPE cartridge with 2 mL MeOH and 2 mL buffer. Prepare whole blood by freezing then thawing, dilute with an equal volume of water, centrifuge at 5000 g for 5 min. 1 mL Plasma or whole blood supernatant + 100 μL 10 μg/mL IS in MeCN:water 50:50 + 2 mL MeCN, vortex for 15 s, centrifuge at 1500 g for 10 min, add the supernatant to the SPE cartridge, allow to dry under vacuum for 1 min, wash with two 2 mL portions of buffer, wash with two 2 mL portions of MeOH:water 50:50, elute with four 750 μL portions of ethyl acetate:acetic acid 98:2. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 (plasma) or 100 (whole blood) μL mobile phase, inject a 50 μL aliquot. (Buffer was 1 g/L potassium bicarbonate.)

HPLC VARIABLES

Column: 250 × 4 5 μm Lichrospher 60 RP select B C8

Mobile phase: MeCN:water 35:65 containing 1% triethylamine, adjusted to pH 4 with orthophosphoric acid

Flow rate: 1.1

Injection volume: 50

Detector: F ex 300 em 375

CHROMATOGRAM

Retention time: 6.8

Internal standard: N-tert-butyl-3-hydroxy-(1,3-dichloro-6-trifluoromethyl-9-phenanthryl)propionamide hydrate (S76,395-0, Aldrich) (12.0)

Limit of detection: 10.8 ng/mL (whole blood), 6.1 ng/mL (plasma)

Limit of quantitation: 14.8 ng/mL (whole blood), 12.4 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: chloroquine, mefloquine, proguanil, pyrimethamine, quinine, sulfadoxine

KEY WORDS
whole blood; plasma; SPE

REFERENCE
Gaillard,Y.; Prévosto,J.-M.; Cheminel,V.; Soares,O.; Chaulet,J.-F. New solid-phase extraction for an improved high-performance liquid chromatographic procedure for the quantitation of halofantrine and monodesbutylhalofantrine in blood or plasma, *J.Chromatogr.B*, **1995**, *668*, 315–321.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1 mL MeCN + 200 μL 2 μg/mL IS in MeCN, vortex for 2 min, centrifuge, add 8 mL MTBE, vortex for 2 min, centrifuge at 700 g for 5 min. Remove 8 mL of the upper organic layer and add it to 100 μL 5 mM HCl in MeCN, evaporate to dryness under a stream of nitrogen at 35°, reconstitute the residue in 200 μL MeCN, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Aquapore (Analytical Biosystems)
Column: 250 × 4.6 5 μm Ultrasphere C8
Mobile phase: MeCN:water 75:25 containing 0.2% sodium dodecyl sulfate and 0.2% glacial acetic acid
Flow rate: 1.5
Injection volume: 25
Detector: UV 257

CHROMATOGRAM
Retention time: 7.8
Internal standard: 2,4-dichloro-6-trifluoromethyl-9-[1-(2-(dibutylamino)ethyl]pehnanthrene-methanol hydrochloride (10.4)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; dog; pharmacokinetics

REFERENCE
Humberstone,A.J.; Currie,G.J.; Porter,C.J.H.; Scanlon,M.J.; Charman,W.N. A simplified liquid chromatography assay for the quantitation of halofantrine and desbutylhalofantrine in plasma and identification of a degradation product of desbutylhalofantrine formed under alkaline conditions, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 265–272.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 258.2

CHROMATOGRAM
Retention time: 22.993

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Adjust pH of 1.75 mL microsomal incubation to pH 9 with 100 mM NaOH, add 3.5 mL n-hexane:diethyl ether 70:30, shake mechanically for 15 min, centrifuge at 2500 g for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 10 μm Chiralcel OD cellulose tris-3,5-dimethylphenylcarbamate
Column: 250 × 4.6 10 μm Chiralcel OD cellulose tris-3,5-dimethylphenylcarbamate
Mobile phase: n-Hexane:isopropanol:diethylamine 90:10:0.1
Flow rate: 0.3
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 27 (+), 31.5 (-)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver; chiral

REFERENCE
Terefe,H.; Blaschke,G. Direct determination of the enantiomers of halofantrine and its pharmacologically active metabolite N-desbutylhalofantrine by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *615*, 347–351.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve a sample in MeOH to a concentration of about 1 mg/mL, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spherisorb SCX
Mobile phase: MeOH:water 80:20 containing 20 mM ammonium formate and 2.3 mL/L trifluoroacetic acid
Flow rate: 1

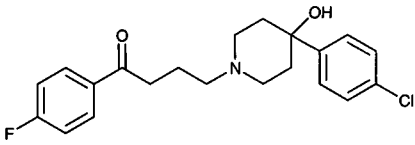
Injection volume: 1-10
Detector: UV 270

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: cimetidine, clomipramine, haloperidol, minoxidil, reserpine, verapamil

REFERENCE
Law,N.; Appleby,J.R.G. Re-evaluation of strong cation-exchange high-performance liquid chromatography for the analysis of basic drugs, *J.Chromatogr.A*, **1996**, *725*, 335–341.

Haloperidol



Molecular formula: $C_{21}H_{23}ClFNO_2$
Molecular weight: 375.87
CAS Registry No.: 52-86-8, 74050-97-8 (decanoate)
Merck Index: 4629
Lednicer No.: 1 306

SAMPLE
Matrix: blood
Sample preparation: Mix 0.5 mL plasma with 25 μL 1 μg/mL IS in MeOH . Vortex for 30 s, add 5 mL ether and 3 mL 100 mM HCl, vortex for 1 min, centrifuge at 3000 rpm for 3 min. Remove the aqueous phase, add 7 mL chloroform (Caution! Chloroform is a carcinogen!) and 500 μL 1 M NaOH. Shake the mixture for 2 min, remove the chloroform phase, evaporate it to dryness under vacuum at 45°. Reconstitute the residue with 500 μL mobile phase, vortex for 1 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeOH:buffer 55:45 (Buffer was water containing 200 mM ammonium acetate, adjusted to pH 7.1-7.3 with acetic acid.)
Column temperature: 38
Flow rate: 1.5
Injection volume: 20
Detector: UV 249

CHROMATOGRAM
Retention time: 6.3
Internal standard: diazepam (5.1)
Limit of detection: 5 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
El-Sayed,Y.M.; Khidr,S.H.; Niazy,E.M. High-performance liquid chromatographic assay for the determination of haloperidol in plasma, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 125–134.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 500 μL 600 mM pH 10 sodium carbonate/bicarbonate buffer + 8 mL heptane:isoamyl alcohol 98:2, shake at 250 cycles/min for 5 min, centrifuge at 1500 g for 10 min. Freeze the aqueous layer, evaporate the heptane layer to dryness under a gentle stream of nitrogen at 60°. Dissolve the residue in 75 μL mobile phase, inject a 65 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 LiChroCart
Mobile phase: MeOH:40 mM pH 7.0 ammonium acetate buffer 90:10
Flow rate: 1
Injection volume: 65
Detector: UV 280

CHROMATOGRAM
Retention time: 5.10
Internal standard: haloperidol

OTHER SUBSTANCES
Simultaneous: amitriptyline, citalopram, chlorprothixene, clomipramine, clozapine, desipramine, desmethylcitalopram, desmethylclomipramine, desmethylsertraline, diltiazem, fluoxetine, fluphenazine, 10-hydroxyamitriptyline, 8-hydroxyclomipramine, 8-hydroxydesmethylclomipramine, 10-hydroxynortriptyline, hydroxyzine, imipramine, methotrimeprazine sulfoxide, mianserine, norfluoxetine, nortriptyline, paroxetine, perphenazine, sertraline, zuclopenthixol
Noninterfering: carbamazepine, clonazepam, flunitrazepam, nitrazepam, oxazepam, oxcarbazepine

KEY WORDS
serum; haloperidol is IS

REFERENCE
Olesen,O.V.; Linnet,K. Simplified high-performance liquid chromatographic method for determination of risperidone and 9-hydroxyrisperidone in serum from patients comedicated with other psychotropic drugs, *J.Chromatogr.B*, **1997**, *698*, 209–216.

SAMPLE
Matrix: blood
Sample preparation: After each extraction or wash step, centrifuge the sample at 2000 g for 5 min. 2 mL Plasma + 50 μL 500 ng/mL IS + 500 μL 2 M NaOH + 8 mL n-hexane:isoamyl alcohol 99:1, extract gently for 20 min on rotator in a horizontal position, centrifuge at 2000 g for 5 min. Extract the organic layer with 1 mL 200 mM HCl for 15 min by vigorous shaking, discard the organic layer, wash the aqueous layer with 5 mL n-hexane:isoamyl alcohol 99:1, discard the organic layer. Add 500 μL 2 M NaOH, extract with 7 mL n-hexane:isoamyl alcohol 99:1 for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 50 μL mobile phase, inject a 1 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 5 μm Nucleosil C18
Mobile phase: MeCN:2 mM ammonium formate 45:55 adjusted to pH 3.0 with formic acid
Flow rate: 50 μL/min
Injection volume: 1
Detector: MS, API 100 Sciex, electrospray, nitrogen as the nebulizing and curtain gas, orifice voltages 20, 60, 120, m/z 376.2

CHROMATOGRAM
Retention time: 6.6
Internal standard: chlorohaloperidol (8.7)
Limit of detection: 75 pg/mL
Limit of quantitation: 100 pg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE

Hoja,H.; Marquet,P.; Verneuil,B.; Lotfi,H.; Dupuy,J.L.; Pénicaut,B.; Lachâtre,G. Determination of haloperidol and its reduced metabolite in human plasma by liquid chromatography-mass spectrometry with electrospray ionization, *J.Chromatogr.B*, **1997**, *688*, 275–280.

SAMPLE

Matrix: blood

Sample preparation: 10 mL Plasma or whole blood + 1 mL 1 M NaOH, extract twice with 10 mL hexane for 30 min. Remove the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, vortex for 1 min, centrifuge. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (It is implied, but not explicitly stated in the paper, that this extraction procedure works for this compound.)

HPLC VARIABLES

Column: 10 μm Micropak CN (Varian)
Mobile phase: MeCN:20 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 5.1
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: acetophenazine, amitriptyline, benztropine, butaperazine, carphenazine, chlorpromazine, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, promazine, promethazine, thioridazine, thiothixene, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine
Interfering: fluphenazine

KEY WORDS

plasma; whole blood

REFERENCE

Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 100 μL 1 μg/mL loxapine in isopropanol:diethylamine 99.9:0.1 + 250 μL 25% potassium carbonate containing 0.1% diethylamine + 5 mL hexane: isoamyl alcohol 97:3, vortex for 30 s, centrifuge at 500 g for 3 min. Remove the organic layer and add it to 100 μL 250 mM HCl, vortex for 30 s, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: 50 × 4.6 40 μm C8 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil C8
Mobile phase: MeCN:water:diethylamine:85% phosphoric acid 53.3:45.1:1:0.4, pH adjusted to 7.2 with NaOH or phosphoric acid
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: k' 3.34
Internal standard: loxapine (k' 7.18)

OTHER SUBSTANCES
Extracted: amitriptyline, chlordiazepoxide, chlorpromazine, desipramine, desmethldiazepam, desmethylchlordiazepoxide, desmethyldoxepin, diazepam, doxepin, fluphenazine, imipramine, oxazepam
Noninterfering: molindone, perphenazine, trifluoperazine
Interfering: nortriptyline, thiothixene

KEY WORDS
plasma

REFERENCE
Kiel,J.S.; Abramson,R.K.; Morgan,S.L.; Voris,J.C. A rapid high performance liquid chromatographic method for the simultaneous measurement of six tricyclic antidepressants, *J.Liq.Chromatogr.*, **1983**, *6*, 2761–2773.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut C18 SPE cartridge with 2 mL MeOH, 2 mL water, 2 mL MeOH, and 2 mL water. 1 mL Plasma or serum + 2 mL 100 mM pH 9.0 borate buffer, vortex for 10 s. Add to the SPE cartridge, wash with 5 mL water, elute with 1.6 mL 200 mM HCl in MeOH. Evaporate the eluate to dryness at 80°, reconstitute in 50 μL 600 ng/mL triazolam in mobile phase, centrifuge at 3000 rpm for 5 min, inject a 15 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 X 4 10 μm Hitachi ODS-3056
Mobile phase: MeCN:THF:1% acetate:triethylamine 28.2:1.9:69.5:0.4
Column temperature: 50
Flow rate: 1.6
Injection volume: 15
Detector: UV 245

CHROMATOGRAM
Retention time: 8
Internal standard: triazolam (14)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites, amitriptyline, carpipramine, chlocapramine, clomipramine, chlorpromazine, clorazepam, diazepam, estazolam, flunitrazepam, fluphenazine, haloxazolam, imipramine, levomepromazine, maprotiline, moperone, nitrazepam, perphenazine, promethazine, triazolam, trifluperidol, trimipramine
Interfering: amoxapine

KEY WORDS
plasma; serum; SPE

REFERENCE
Hayakari,M.; Hashimoto,Y.; Kita,T.; Murakami,S. A rapid and simplified extraction of haloperidol from plasma or serum with Bond Elut C18 cartridge for analysis by high performance liquid chromatography, *Forensic Sci.Int.*, **1987**, *35*, 73–81.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 250 μL di-iso-propyl ether:n-butyl alcohol 7:3 containing 400 ng/mL minaprine, centrifuge 2 min, shake, centrifuge 5 min, inject 50 μL aliquot of top organic layer.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Brownlee cyano spheri-5
Column: 250 × 4.6 5 μm Altex ultrasphere cyano
Mobile phase: MeCN:THF:water:2 M ammonium formate (pH 4.0) 700:100:195:5

Column temperature: 20
Flow rate: 1.5
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 6
Internal standard: minaprine (5.5)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Also analyzed: diltiazem, nortriptyline, amitriptyline, haloperidol, desipramine, imipramine, clomipramine, propafenone, amiodarone, verapamil

KEY WORDS
serum

REFERENCE
Mazzi,G. Simple and practical high-performance liquid chromatographic assay of some tricyclic drugs, haloperidol, diltiazem, verapamil, propafenone, and amiodarone, *Chromatographia*, **1987**, *24*, 313–316.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 40 μL 1 μg/mL chlorohaloperidol in MeOH + 2 mL pH 11 Normex buffer, vortex for 1 min, add to a 3 mL Extrelut SPE cartridge, elute with diethyl ether. Evaporate the eluate to dryness under a stream of air at 40°, reconstitute the residue in 100 μL 10 mM HCl, vortex, add 2 mL hexane, shake on a whirlmixer for 20 s, centrifuge at 2800 g for 5 min, inject a 20-40 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM KH_2PO_4:water 45:50:5
Flow rate: 0.8
Injection volume: 20-40
Detector: UV 220

CHROMATOGRAM
Retention time: 9
Internal standard: chlorohaloperidol (11)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: alimemazine, amisulpride, amitriptyline, biperiden, caffeine, carbamazepine, clobazam, clomipramine, clorazepate, cyamemazine, desipramine, diazepam, ethybenztropine, ethyl loflazepate, flunitrazepam, imipramine, levomepromazine, loflazepate, lorazepam, nordiazepam, oxazepam, sulpride, sultopride, tiapride, triazolam, trihexiphenidyl, trimipramine, tropazepine, viloxazine
Noninterfering: heptaminol, meprobamate
Interfering: nortriptyline

KEY WORDS
SPE; plasma

REFERENCE
Cahard,C.; Rop,P.P.; Conquy,T.; Viala,A. High-performance liquid chromatographic analysis of haloperidol and hydroxyhaloperidol in plasma after solid-phase extraction, *J.Chromatogr.*, **1990**, *532*, 193–202.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL 200 ng/mL IS in MeOH + 1 mL 50 mM pH 10 borate buffer, vortex briefly, add to an Extrelut 3 SPE cartridge, let stand for 5 min, elute with 15 mL hexane:dichloromethane 50:50. Add the eluate to 3 mL 50 mM sulfuric acid, mix for 10 min, centrifuge at 3000 g for 10 min. Remove the aqueous layer and add it to 6 mL hexane: dichloromethane 50:50, wash for 5 min, centrifuge. Make the aqueous layer basic with 150 μL 28% ammonia, extract twice with 3 mL hexane:dichloromethane 50:50. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Spherisorb cyano
Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:buffer 60:40 (Buffer was 50 mM KH_2PO_4 adjusted to pH 6.5 with 28% ammonia.)
Flow rate: 1
Injection volume: 20
Detector: E, 5100 A Coulochem, 5020 guard cell 1.00 V, 5011 analytical cell, detector 1 0.55 V, detector 2 0.80 V, output of detector 2 is monitored

CHROMATOGRAM

Retention time: 21.1 (haloperidol), 14.9 (reduced haloperidol)
Internal standard: methylrisperidone (R68808) (14.3)

OTHER SUBSTANCES

Extracted: chlorpromazine, clomipramine, cyamemazine, desipramine, droperidol, flunitrazepam, imipramine, pipamperone, risperidone, trihexyphenidyl
Noninterfering: alprazolam, bromazepam, carbamazepine, chlorazepate, diazepam, diphenylhydantoin, estazolam, ethylbenzatropine, oxazepam, phenobarbital, triazolam, valproic acid

KEY WORDS

plasma; SPE

REFERENCE

Le Moing,J.P.; Edouard,S.; Levron,J.C. Determination of risperidone and 9-hydroxyrisperidone in human plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1993**, *614*, 333–339.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 40 ng/mL chlorohaloperidol in MeCN + 500 μL saturated sodium carbonate, mix well, add 7 mL pentane:dichloromethane 90:10, shake in a Vibrax shaker for 10 min, centrifuge at 18° at 1725 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 150 μL MeCN, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:MeOH:40 mM ammonium acetate 86:6:8
Column temperature: 40
Flow rate: 0.8
Injection volume: 150
Detector: E, ESA Coulochem Model 5100A, Model 5011 porous graphite analytical cell, electrode 1 0.6 V (screening), electrode 2 0.95 V (detection), Model 5020 guard cell 1 V (between pump and injector)

CHROMATOGRAM

Retention time: 12
Internal standard: chlorohaloperidol (11)
Limit of quantitation: 0.1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Noninterfering: acetaminophen, benztropine, clonazepam, clozapine, fluphenazine, ibuprofen, lorazepam, pseudoephedrine, trihexiphenidyl

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Aravagiri,M.; Marder,S.R.; Van Putten,T.; Marshall,B.D. Simultaneous determination of plasma haloperidol and its metabolite reduced haloperidol by liquid chromatography with electrochemical detection. Plasma levels in schizophrenic patients treated with oral or intramuscular depot haloperidol, *J.Chromatogr.B*, **1994**, *656*, 373–381.

SAMPLE
Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM
Retention time: 3.4 (haloperidol), 2.5 (reduced haloperidol)
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, diazepam, encainide, fluoxetine, flurazepam, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, norfluoxetine, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trimipramine, verapamil
Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin
Interfering: dextromethorphan, diphenhydramine, doxepin, fentanyl, flecainide, nordoxepin, trazodone

KEY WORDS
plasma; SPE

REFERENCE
Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 221

CHROMATOGRAM

Retention time: 6.08

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Bond Elut Certify SPE cartridge with 2 mL MeOH and 2 mL 100 mM pH 6.0 phosphate buffer, do not allow to dry. 1 mL Blood + 6 mL 100 mM pH 6.0 phosphate buffer, vortex, sonicate, centrifuge, add the supernatant to the SPE cartridge, wash with water, wash with 1 mM pH 3.3 acetic acid, dry by suction, wash with 2 mL acetone: chloroform 50:50, elute with 3 mL ethyl acetate:ammonia 98:2. Evaporate the eluate under a stream of nitrogen at 40°, reconstitute the residue in 50 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pack C18
Mobile phase: Gradient. MeOH:50 mM ammonium acetate 65:35 for 1 min, to 75:25 over 4 min, maintain at 75:25 for 20 min (Mix column effluent with 50 mM ammonium acetate pumped at 0.5 mL/min.)
Flow rate: 0.6
Injection volume: 10
Detector: MS, Finnigan MAT TSQ 700 tandem quadrupole, MAT TSP-2 interface, thermospray, selective reaction monitoring m/z 376-165, collision offset -27 V, repeller 100 V, vaporizer 130°, source 200°, filament on 200 μA, argon 2.5 mTorr, multiplier 1500 V, dynode 15 kV, scan time 1.20 s, MSMSC factor 10

CHROMATOGRAM
Retention time: 5.00
Limit of detection: 50 pg

OTHER SUBSTANCES
Extracted: benperidol, dextromoramide, droperidol, methadone, penfluridol, pimozide, pipamperidone, propoxyphene (dextropropoxyphene)

KEY WORDS
SPE; LC/MS

REFERENCE
Verweij,A.M.; Hordijk,M.L.; Lipman,P.J. Quantitative liquid chromatographic thermospray-tandem mass spectrometric analysis of some analgesics and tranquilizers of the methadone, butyrophenone, or diphenylbutylpiperidine groups in whole blood, *J.Anal.Toxicol.*, **1995**, *19*, 65–68.

SAMPLE
Matrix: blood, gastric contents, tissue, urine
Sample preparation: 1 mL Blood, urine, or gastric contents or 1 g tissue homogenate + 500 μL buffer + 8 mL n-hexane:ethyl acetate 70:30, mix on a rotary mixer for 10 min, centrifuge at 3000 g for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL 12.5 mM NaOH in MeOH:water 50:50, inject a 50 μL aliquot. (Buffer was 13.8 g potassium carbonate in 100 mL water, pH adjusted to 9.5 with concentrated HCl.)

HPLC VARIABLES
Guard column: 4 × 4 30 μm LiChrocart Aluspher RP-select B (Merck)
Column: 125 × 4 5 μm Aluspher RP-select B (Merck)
Mobile phase: Gradient. A was 12.5 mM NaOH in MeOH. B was 12.5 mM NaOH in water. A:B 10:90 for 5 min, to 90:10 over 15 min, maintain at 90:10 for 5 min, return to initial conditions over 1 min, re-equilibrate for 5 min.
Flow rate: 1
Injection volume: 50
Detector: UV 230, 254

CHROMATOGRAM
Retention time: 17

OTHER SUBSTANCES

Extracted: alprenolol, amitriptyline, bromazepam, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, desipramine, diazepam, flunitrazepam, nitrendipine, nordiazepam, nortriptyline, pindolol, zolpidem

Also analyzed: acebutolol, acetaminophen, alprazolam, amphetamine, atenolol, betaxolol, brotizolam, caffeine, camazepam, captopril, chloroquine, clobazam, clomipramine, clothiapine, clotiazepam, cloxazolam, cocaine, codeine, diclofenac, dihydralazine, dihydrocodeine, dihydroergotamine, diphenhydramine, domperidone, doxepin, droperidol, ergotamine, ethyl loflazepate, fenethylline, fluoxetine, flupentixol, flurazepam, furosemide, gliclazide, hydrochlorothiazide, hydroxyzine, ibuprofen, imipramine, ketazolam, loprazolam, lorazepam, lormetazepam, maprotiline, medazepam, mepyramine, methadone, methaqualone, methyldopa, methylphenidate, metoclopramide, metoprolol, mexiletine, mianserin, midazolam, minoxidil, morphine, nadolol, nitrazepam, oxprenolol, papaverine, pentazocine, phenprocoumon, phenylbutazone, pipamperone, piritramide, practolol, prazepam, prazosin, promazine, promethazine, propoxyphene, propranolol, prothipendyl, quinine, sotalol, sulpride, thioridazine, trazodone, triazolam, trimipramine, tripelennamine, tyramine, verapamil, yohimbine

REFERENCE

Lambert,W.E.; Meyer,E.; De Leenheer,A.P. Systematic toxicological analysis of basic drugs by gradient elution of an alumina-based HPLC packing material under alkaline conditions, *J.Anal.Toxicol.*, **1995**, *19*, 73–78.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 4.80
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, imipramine, loxapine, maprotiline, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

Interfering: meperidine, norpropoxyphene, northiaden

KEY WORDS

serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 14.415

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out capsule contents equivalent to 400 μg haloperidol, dissolve in 30 mL MeOH, add 1 mL 100 μg/mL isothipendyl hydrochloride in MeOH, filter (paper), wash filter with MeOH, make up filtrate to 50 mL with MeOH. Dilute a 1 mL aliquot to 10 mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Econosphere C18
Mobile phase: MeOH:water:triethylamine 80:20:0.15, pH adjusted to 7.8 with phosphoric acid
Flow rate: 1.8
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4
Internal standard: isothipendyl (7)
Limit of quantitation: 800 ng/mL

OTHER SUBSTANCES
Simultaneous: propantheline bromide

KEY WORDS
capsules

REFERENCE
Sane,R.T.; Ghadge,J.K.; Jani,A.B.; Vaidya,A.J.; Kotwal,S.S. Simultaneous high-performance liquid chromatographic determination of haloperidol with propantheline bromide, nalidixic acid with phenazopyridine hydrochloride, and dipyridamole with aspirin in combined dosage (forms), *Indian Drugs*, **1992**, *29*, 240–244.

SAMPLE
Matrix: formulations
Sample preparation: 100 μL Injection solution + 400 μL 2.5 μg/mL haloperidol in water, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Brownlee C18
Mobile phase: MeCN:MeOH:10 mM NaH_2PO_4 24:31:45, pH adjusted to 5.0 with 2 M KOH
Flow rate: 1.7
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 9.74
Internal standard: haloperidol

OTHER SUBSTANCES
Simultaneous: fentanyl, sufentanil

KEY WORDS
injections; haloperidol is IS

REFERENCE
Dewell,W.M.,Jr.; Khandaghabadi,M.; D'Souza,M.J.; Solomon,H.M. High-performance liquid chromatographic determination of fentanyl and sufentanil returned from the operating room, *Am.J.Hosp.Pharm.*, **1993**, *50*, 2374–2375.

SAMPLE
Matrix: hair
Sample preparation: Wash hair in water, rinse 3 times with MeOH, dry, weigh. 5-25 mg Washed hair + 1 mL 1 M NaOH, heat at 70° for 30 min, adjust pH to 9.5-10. 1 mL Extract + 1 μg protriptyline + 1 mL water + 1 mL 200 mM sodium carbonate buffer, mix, extract with hexane: butanol 95:5 for 20 min. Remove the organic layer and add it to 100 μL 0.2% orthophosphoric acid, mix for 20 min, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Newguard RP-18
Column: 100 × 4.6 Spheri-5 RP-C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 1.2 L 100 mM pH 7.0 NaH_2PO_4 + 30 mL diethylamine.)
Flow rate: 2
Injection volume: 30
Detector: UV 214

CHROMATOGRAM
Retention time: 2.5
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: amitriptyline, clomipramine, desipramine, dothiepin, doxepin, imipramine, mianserin, nortriptyline

KEY WORDS
may be interferences

REFERENCE
Couper,F.J.; McIntyre,I.M.; Drummer,O.H. Extraction of psychotropic drugs from human scalp hair, *J.Forensic Sci.*, **1995**, *40*, 83–86.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve a sample in MeOH to a concentration of about 1 mg/mL, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spherisorb SCX
Mobile phase: MeOH:water 80:20 containing 20 mM ammonium formate and 2.3 mL/L trifluoroacetic acid
Flow rate: 1
Injection volume: 1-10
Detector: UV 270

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Simultaneous: cimetidine, clomipramine, halofantrine, minoxidil, reserpine, verapamil

REFERENCE
Law,N.; Appleby,J.R.G. Re-evaluation of strong cation-exchange high-performance liquid chromatography for the analysis of basic drugs, *J.Chromatogr.A*, **1996**, *725*, 335–341.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.0

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine,

ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 2 5 μm Hypersil CPS
Column: 250 × 4.6 5 μm Hypersil CPS-5
Mobile phase: MeCN:10 mM pH 5.4 ammonium acetate 67:33
Flow rate: 1
Injection volume: 100
Detector: UV 220, UV 245

CHROMATOGRAM

Retention time: 13 (haloperidol), 11.5 (reduced haloperidol)
Internal standard: pirenzepine (8)
Limit of detection: 150 pmole

OTHER SUBSTANCES

Simultaneous: metabolites

KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Tomlinson,A.J.; Benson,L.M.; Landers,J.P.; Scanlan,G.F.; Fang,J.; Gorrod,J.W.; Naylor,S. Investigation of the metabolism of the neuroleptic drug haloperidol by capillary electrophoresis, *J.Chromatogr.A*, **1993**, *652*, 417–426.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)

Flow rate: 0.5-1
Detector: UV 215, 241

CHROMATOGRAM
Retention time: 3.0
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES
Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

REFERENCE
Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primi-

done, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 11.10 (A), 6.17 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 8.91

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. Homogenize kidney with a kitchen grinder. Weigh out a 5 g sample and add 20 mL MeCN with continuous gentle mixing, mix vigorously on a vibromixer at 1500 rpm for 30 s, sonicate for 2 min, centrifuge at 4000 g for 5 min. Mix 7.5 mL sample extract and 40 mL 10% NaCl and add to SPE cartridge, wash with 1 mL 10 mM sulfuric acid, wash with 2 mL air, elute with 2 mL acidic MeCN. Place eluate in a washed tube and evaporate to 300 μL at 70° under a stream of nitrogen, mix gently, add 1 mL n-hexane, mix on a vibromixer for 30 s, centrifuge at 2000 g, inject a 50 μL aliquot of the aqueous phase. (Acidic MeCN was 1 mL 50 mM sulfuric acid and 100 mL MeCN. The washed tube was prepared by rinsing with concentrated ammonia, water, and acetone and drying under a stream of nitrogen.)

HPLC VARIABLES
Guard column: 10 × 2.1 37-50 μm Bondapak C18
Column: 300 × 3.9 Bondapak C18
Mobile phase: MeCN:water 55:45 containing 2.46 g/L anhydrous sodium acetate, pH adjusted to 6.5 with acetic acid
Flow rate: 1.2
Injection volume: 50

Detector: UV 240

CHROMATOGRAM
Retention time: 8
Limit of detection: 2 ng/g

OTHER SUBSTANCES
Extracted: azaperol, carazolol, acepromazine, xylazine, azaperone, propiomazine, chlorpromazine

KEY WORDS
SPE; pig; kidney

REFERENCE
Keukens,H.J.; Aerts,M.M.L. Determination of residues of carazolol and a number of tranquilizers in swine kidney by high-performance liquid chromatography with ultraviolet and fluorescence detection, *J.Chromatogr.*, **1989**, *464*, 149–161.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Bond-Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Cut pig kidney or liver into small pieces and homogenize. 5 g Homogenate + 10 mL MeCN, shake, vortex for 30 s, sonicate for 3 min, vortex for 30 s, sonicate for 3 min, centrifuge at 10000 g for 20 min. Add 7.5 mL supernatant + 40 mL 10% NaCl to the SPE cartridge at about 1 mL/min, do not allow cartridge to dry out, wash with 850 μL 10 mM sulfuric acid, dry with air, elute with 3.5 mL acidic MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μL 10 mM sulfuric acid, vortex briefly, add 1 mL hexane, vortex for 30 s, centrifuge at 2000 g for 5 min, inject an aliquot of the aqueous layer. (Acidic MeCN was 1 mL 50 mM sulfuric acid in 100 mL MeCN.)

HPLC VARIABLES
Guard column: Hypersil 5 μm SAS C1
Column: 250 mm long 5 μm Hypersil SAS C1
Mobile phase: MeCN:water 50:50 containing 0.77 g/L ammonium acetate
Flow rate: 2
Detector: E, ESA Model 5100A Coulochem, first electrode +0.4 V, second electrode (which was monitored) +0.7 V, Model 5020 guard cell after pump but before injector at +0.75 V

CHROMATOGRAM
Retention time: 12.5
Limit of detection: 2 ng/g

OTHER SUBSTANCES
Extracted: azaperol, acepromazine, carazolol, azaperone, xylazine, propriomazine, chlorpromazine

KEY WORDS
SPE; pig; kidney; liver

REFERENCE
Rose,M.D.; Shearer,G. Determination of tranquilisers and carazolol residues in animal tissue using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1992**, *624*, 471–477.

Halothane

Molecular formula: $C_2HBrClF_3$
Molecular weight: 197.38
CAS Registry No.: 151-67-7
Merck Index: 4634

SAMPLE
Matrix: solutions
Sample preparation: Mix 50 μL phosphate buffer containing isoflurane and 50 μL 0.05 mM toluene in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: RCSS Guard-Pak μBondapak C18 precolumn cartridge
Column: 100 × 8 4 μm Nova-Pak C18 Radial Compression Module
Mobile phase: MeOH:water 50:50
Flow rate: 3.5
Injection volume: 20
Detector: UV 203

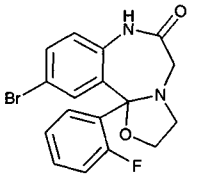
CHROMATOGRAM
Retention time: 6
Internal standard: toluene (12)
Limit of detection: 0.001 mM

OTHER SUBSTANCES
Simultaneous: enflurane, isoflurane

KEY WORDS
buffer

REFERENCE
Janicki,P.K.; Erskine,W.A.R.; James,M.F.M. High-performance liquid chromatographic method for the direct determination of the volatile anaesthetics halothane, isoflurane and enflurane in water and in physiological buffer solutions, *J.Chromatogr.*, **1990**, *518*, 250–253.

Haloxazolam



Molecular formula: $C_{17}H_{14}BrFN_2O_2$
Molecular weight: 377.21
CAS Registry No.: 59128-97-1
Merck Index: 4635

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 20 μL 20 μg/mL IS + 200 μL 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate the organic phase under a stream of nitrogen at 40°. Dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-ODS (A) or 100 × 4.6 5 μm Hypersil ODS-C18 (B)
Mobile phase: MeCN:5 mM pH 6 NaH_2PO_4 45:55
Flow rate: 0.65
Injection volume: 20

Detector: UV 254

CHROMATOGRAM
Retention time: 41.5 (A), 100.6 (B)
Internal standard: diazepam (29.8 (A), 77.5 (B))
Limit of quantitation: 50 ng/mL (A)

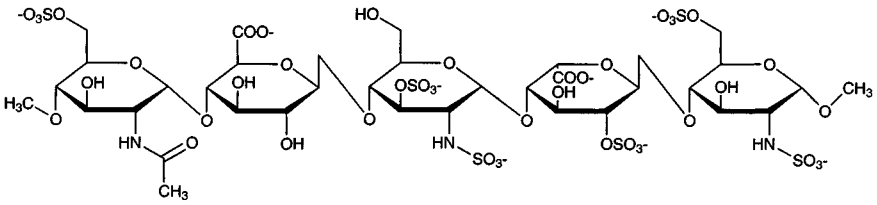
OTHER SUBSTANCES
Extracted: bromazepam, chlordiazepoxide, clonazepam, estazolam, etizolam, flutazolam, lorazepam, nitrazepam, oxazolam, triazolam
Simultaneous: alprazolam
Noninterfering: barbital, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione

KEY WORDS
serum

REFERENCE
Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-μm porous microspherical silica gel, *J.Chromatogr.B*, **1996**, *682*, 173–178.

Heparin

Molecular weight: 6000-30000
CAS Registry No.: 9005-49-6, 9041-08-1 (Na salt)
Merck Index: 4685



SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL 10 M NaOH, shake on a slow rotatory mixer for 5 min, add 5 mL diethyl ether, rotomix 10 min, centrifuge at 700 g for 5 min, repeat extraction. Combine organic layers, evaporate to dryness under a stream of nitrogen at 37°, dissolve in 250 μL mobile phase, inject aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Novapack C18
Mobile phase: MeCN:MeOH:buffer 35:15:50 (Buffer was 50 mM KH_2PO_4 adjusted to pH 3.6 with phosphoric acid.)
Flow rate: 1.6
Injection volume: 50
Detector: E, Waters Model 464 pulsed electrochemical detector, + 1 V versus Ag/AgCl

CHROMATOGRAM
Retention time: 0.99
Limit of detection: 50 pg/mL

OTHER SUBSTANCES
Simultaneous: ethinylestradiol, estrone, estriol, estradiol
Noninterfering: pentobarbital

KEY WORDS
plasma; rabbit

REFERENCE
Fernández,N.; Garcia,J.J.; Diez,M.J.; Terán,M.T.; Sierra,M. Rapid high-performance liquid chromatographic assay of ethynyloestradiol in rabbit plasma, *J.Chromatogr.*, **1993**, *619*, 143–147.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:water:glacial acetic acid 4:84:12 containing 4.84 g/L Trizma, pH 2.3
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7.34
Internal standard: 8-chlorotheophylline (5.29)

OTHER SUBSTANCES
Simultaneous: acetaminophen, caffeine, cefazolin, cimetidine, ergotamine, glutethimide, methamphetamine, propranolol, salicylic acid, sulfamethoxazole, theobromine, theophylline, tobutamide, trimethoprim
Noninterfering: amitriptyline, amobarbital, ampicillin, butabarbital, butalbital, celbenine, chlordiazepoxide, chlorpromazine, clorazepate, desipramine, diazepam, doxepin, ethchlorvynol, fluphenazine, hydroxyzine, ibuprofen, imipramine, isoniazid, lidocaine, mephobarbital, mesoridazine, methaqualone, methyluric acid, naprotyline, nordiazepam, nortriptyline, oxazepam, pentobarbital, perphenazine, phenelzine, phenmetrazine, phenobarbital, phenylbutazone, phenytoin, prednisolone, prednisone, procainamide, prochlorperazine, promazine, promethazine, propoxyphene, protriptyline, pyrilamine, secobarbital, thioridazine, thiothixene, timolol, trazodone, triazolam, trifluoperazine

REFERENCE
Osterloh,J.; Yu,S. Simultaneous ion-pair and partition liquid chromatography of acetaminophen, theophylline and salicylate with application to 500 toxicologic specimens, *Clin.Chim.Acta*, **1988**, *175*, 239–248.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 7.5 Ultropac TSK G 2000 SW (LKB)
Mobile phase: 100 mM NaCl
Flow rate: 0.5
Injection volume: 20
Detector: UV 206

CHROMATOGRAM
Retention time: 15, 21, 29

KEY WORDS
SEC

REFERENCE
de Vries,J.X. Analysis of heparins by size-exclusion and reversed-phase high-performance liquid chromatography with photodiode-array detection, *J.Chromatogr.*, **1989**, *465*, 297–304.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 mg/mL solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: TSK G3000SW and TSK G2000SW in series (Tosoh)
Mobile phase: 500 mM sodium sulfate
Flow rate: 0.5
Injection volume: 20
Detector: UV 234 or RI

KEY WORDS
SEC

REFERENCE
Ahsan,A.; Jeske,W.; Hoppensteadt,D.; Lormeau,J.C.; Wolf,H.; Fareed,J. Molecular profiling and weight determination of heparins and depolymerized heparins, *J.Pharm.Sci.*, **1995**, *84*, 724–727.

SAMPLE
Matrix: urine
Sample preparation: Mix 9 mL urine with 600 μL 5% hexadecylpyridinium chloride, keep at 0° for 4 h. Centrifuge at 2300 g for 15 min, wash the precipitate twice with 1.5 mL 0.1% hexadecylpyridinium chloride. Dissolve the precipitate in 1 mL 2.5 M NaCl. Centrifuge at 2300 g for 15 min. Add 11 mL EtOH:water 85:15 to he supernatant, keep overnight at 0°. Centrifuge at 2300 g for 15 min at 4°. Dry the precipitate under reduced pressure, re-dissolve in 50 μL 200 mM pH 8.0 Tris-HCl buffer. Add 10 μL of an aqueous solution containing 0.1 U chondroitinase ABC, incubate at 37°. for 3 h. Add 250 μL EtOH, keep overnight at 4 dg. Centrifuge at 4° at 2300 g for 15 min. Wash the precipitate with three 1 mL portions of EtOH:water 75:25., dry under reduced pressure. Redissolve in 50 μL 100 mM acetate buffer containing 10 mM pH 7.0 calcium acetate. Mix 10 μL sample with 10 μL 100 mM acetate buffer containing 10 mM pH 7.0 calcium acetate and 30 μL aqueous heparin lyase solution, incubate at 37° for 12 h. Inject a 2 μL aliquot. (The heparin lyase solution contained 4 mU heparin lyase I, 0.4 mU heparin lyase II, and 0.4 mU heparin lyase III.)

HPLC VARIABLES
Column: 150 × 2.0 5 μm TSK gel Amide-80 column (TOSOH, Japan)
Mobile phase: MeCN:water:200 mM pH 7.0 sodium phosphate buffer:3.0 M ammonium chloride 32:10:1:1
Column temperature: 60
Flow rate: 0.4
Injection volume: 2
Detector: F ex 346 em 410 following post-column reaction. The column effluent mixed with 1% 2-cyanoacetamide solution containing 500 mM NaOH pumped at 0.25 mL/min and this mixture flowed through a 10 m × 0.5 mm reaction coil at 110° and a 2 m × 0.25 mm cooling coil to the detector.

CHROMATOGRAM
Retention time: 7.1 (δ UA-GlcNAc), 8.5 (δ UA-GlcNAc6S), 10.5 (δ UA2S-GlcNac6S), 11.0 (δ UA-GlcNS), 8.9 (δ UA2S-GlcNS), 16.1 (δ UA-GlcNS6S), 21.2 (δ UA2s-GlcNS6S)
Limit of detection: 2 pmol

KEY WORDS
post-column reaction

REFERENCE
Toyoda,H.; Nagashima,T.; Hirata,R.; Toida,T.; Imanari,T. Sensitive high-performance liquid chromatographic method with fluorometric detection for the determination of heparin and heparan sulfate in biological samples: application to human urinary heparan sulfate, *J.Chromatogr.B*, **1997**, *704*, 19–24.